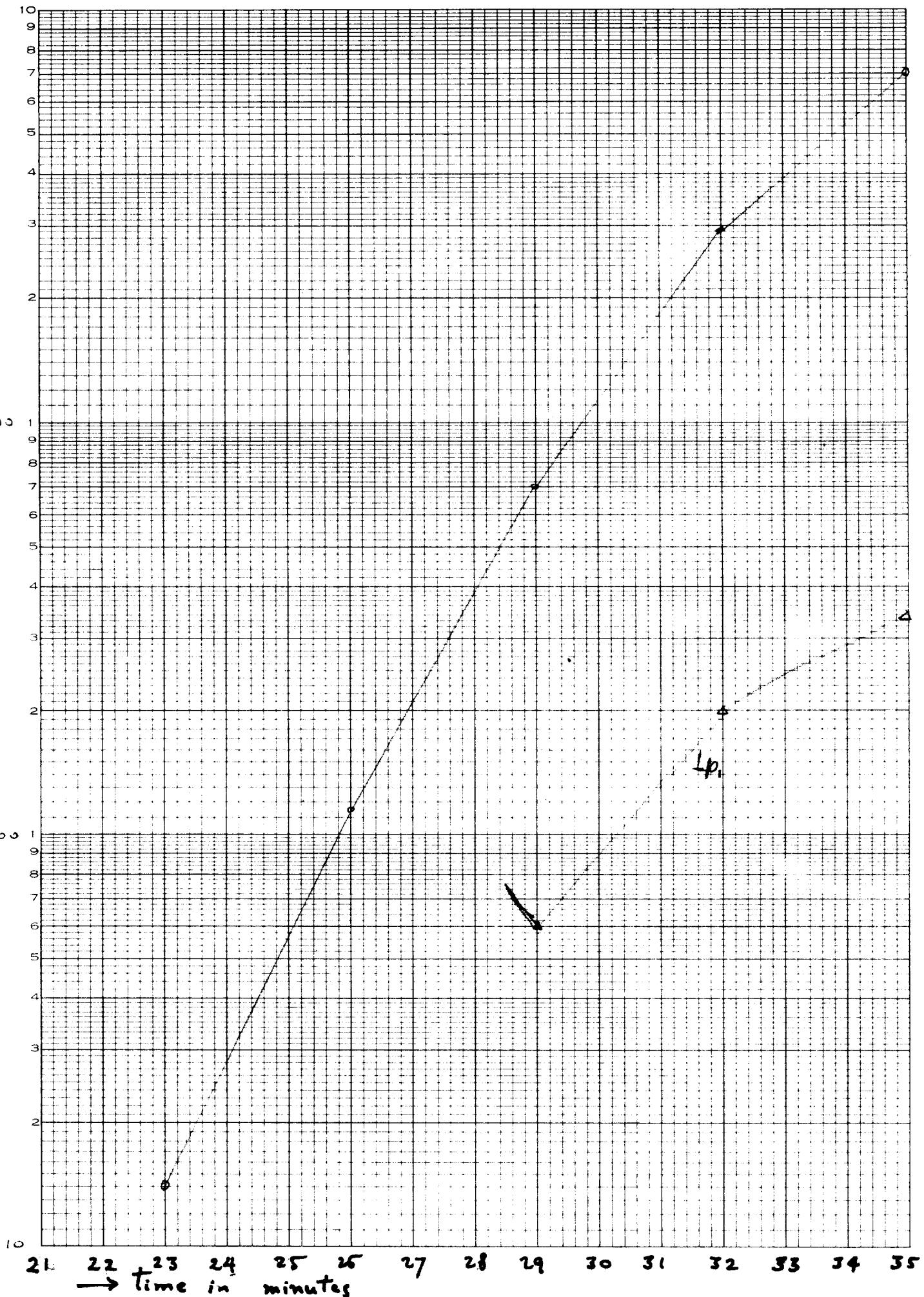


00000 1408/10 gal₂

EUGENE DIETZGEN CO.
PRINTED IN U.S.A.

(O, 340-L310 DIETZGEN GRAPH PAPER
A1 LOGARITHMIC .9 CYCLES X 10 DIVISIONS



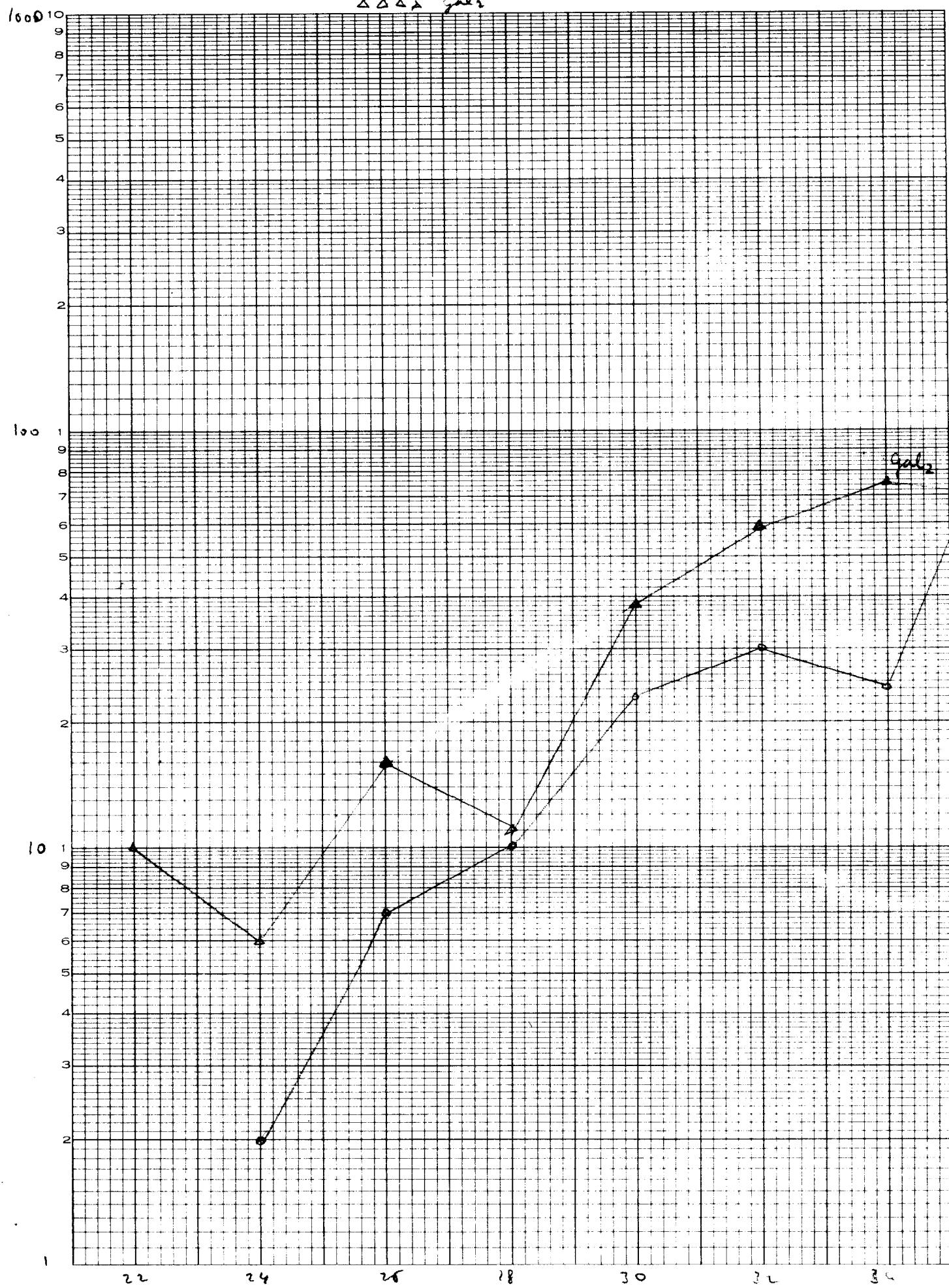
1408/13

0000 Gal,
△△△△ gal₂

partial reversion & f+ of 3870.

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10, 340-L310 DIETZGEN GRAPH PAPER
A1-LOGARITHMIC 3 CYCLES X 10 DIVISIONS



1412

B1

B₂
and C

2

May 1 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T _I	GAL	LAC	T _I	GAL	LAC	T _I	REF.
1	-	-	+	all-	-	+	-	-	+	-
2	-	-	+	-	-	-	-	-	-	-
3	-	-	+	-	-	+	-	-	+	-
4	-	+	-	-	-	-	-	-	-	-
5	-	-	+	-	-	+	-	-	+	-
6	-	-	+	-	-	+	-	-	-	-
7	-	-	+	-	↑	+	-	-	+	-
8	-	-	+	-	.05	+	-	-	+	-
9	-	-	-	-	↓	+	-	-	+	-
0	-	-	-	-	+	+	-	+	+	-
1	-	-	-	-	-	-	-	-	+	-
2	-	-	+	-	-	-	-	-	+	-
3	-	-	+	-	-	+	-	-	-	-
4	-	-	+	-	-	+	-	-	-	-
5	-	-	+	-	-	+	-	-	+	-
6	-	-	+	-	-	-	-	-	-	-
7	-	-	+	-	-	-	-	-	+	-
8	-	-	+	-	-	-	-	-	-	-
9	-	-	+	-	-	+	-	-	+	-
0	-	-	+	-	-	+	+	+	+	-
1	-	-	+	-	-	-	-	-	+	-
2	-	-	-	-	-	+	-	-	+	-
3	-	-	-	-	-	-	-	-	+	-
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9	-	-	+	-	-	-	-	-	+	-
0	-	-	+	-	-	-	-	-	+	-

1408/14

19

1 A : Total count (bimcular)
2
3 B : C. from 10³ Liddell (x empirical factor 14)
4 exp. factor 21).
5

Time	Gal 4		Gal 7		Gal 8		
	A	B	A	B	A	B	
0	0						
22	0		8		0		
24	0		11		6		
25	57		37		55		
28	210	(9)	195		128	(11)	
30	471	(28)	326	(21)	224	(28)	
32	630	45	420	30	427	(27)	
34	994	71	630	45	1190	85	
36	1246	89	1610	115	1176	84	
38	1064	76	2310	165	1666	119	
40	1890	185	1974	141	3080	220	
50	7331	524	6496	464	8596	614	
60	6930	495	6748	482	3430	245	

() between brackets: values used for calculation
of empirical factor; total plate count given in A

19

June 18, 1957

REF:

1408/15.

1

2

Hfr timing of Gal₄, Gal₂, Gal₃.Hfr timing of Gal₄, Gal₂, Gal₃.

5

6

7

8

9

10

1 Exactly as 1408/14, but:

2 ♀ parents were Gal₄, Gal₂, Gal₃, and they were
3 seeded in this order.4 4) ♀ 3 was grown after Gal₄ and Gal₂ were isolated, as
5 had been
6 a substitute for Gal₃ which was supposed to be tested
7 today and was found to be st^s. ♀ 3 was found to be
8 slightly less concentrated than the other two females and
9 three tanks were collected into one, thus reaching 1.5 x conc.
10 for this ♀.

3) Mating mixtures: 5 ml ♂ + 12 ml ♀.

Plate counts. (48 hr) (72 hr)

Time	Gal ₄	Gal ₃	Gal ₂
0	(35), 7	2, 0	13, 0, 0
15	7, 1	0, 3	11, 0, 0
18	2, 9	3, 27, 27	0, 1
20	3, 8	1, 0	23, 15, 5, 2
22	18, 13	2, 3	18, 1, 10
24	138, 112	1, 3	16, 17, 32, 24
26	451, 348	1, 1	8, 17, 68, 51
28	410, 448	0, 1	11, 17, 160, 110
30	t.m.t.b.c.	3, 12	13, 27, 420,
35		28, 25	38, 34
40		55, 67	60,
50		205, 198	
60		391, 356	

Notes: Gal₃: all large colonies; in addition, few (less than 10%) small ones and some extremely small.Gal₂, Gal₄: all colonies small, as in former except

1408/15

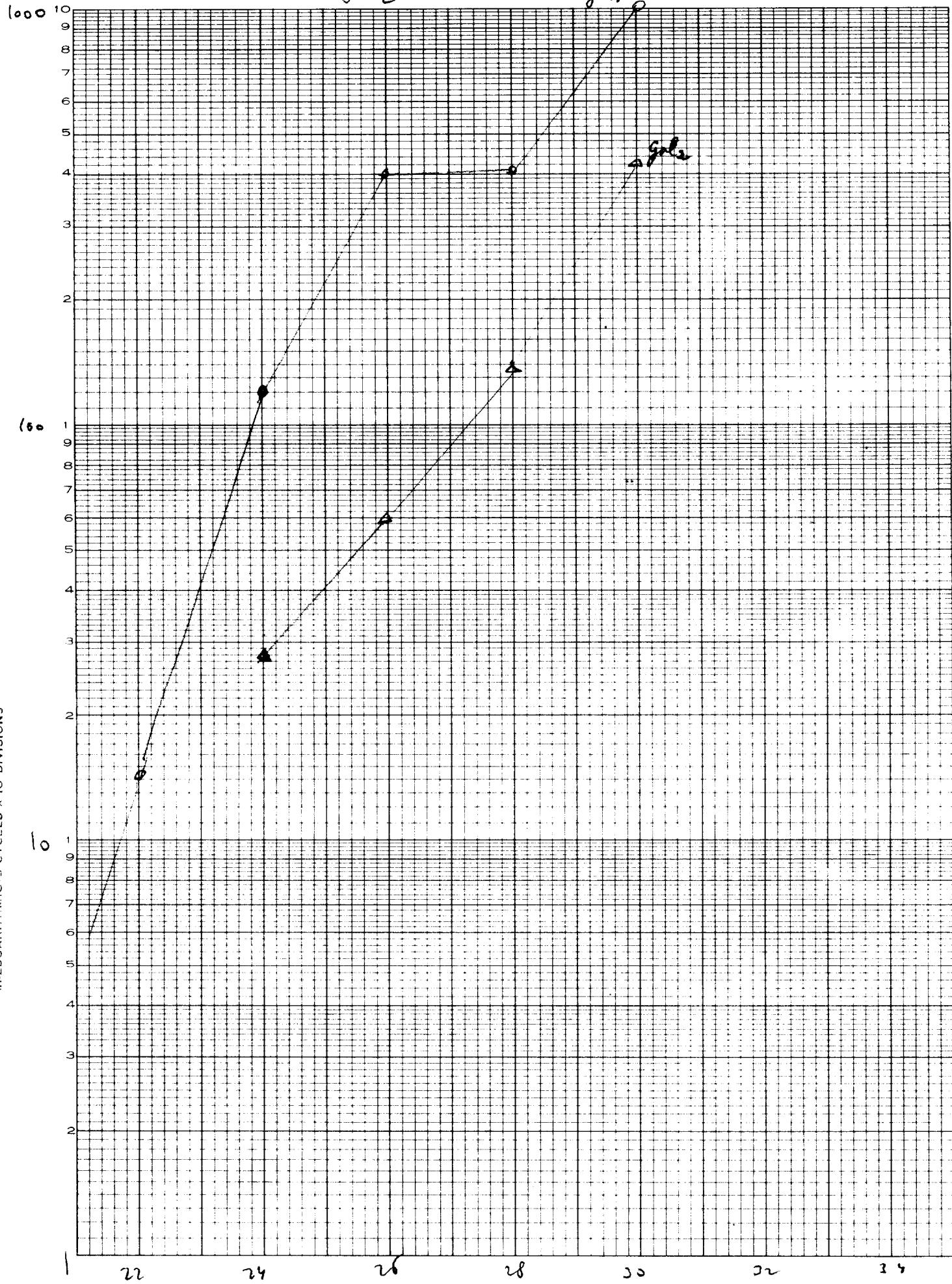
0000 gal 4
△△△ Gal 2

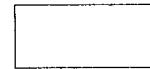
gal 4

Gal 2

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ALLOGRITHMIC. 3 CYCLES X 10 DIVISIONS





19

June 20, 1950.

REF: 1408/16

	1	2	3	4	5	6	7	8	9	10
--	---	---	---	---	---	---	---	---	---	----

1

Hfr₂ timing of Gal_{5,2,1,3}.

2

3

4

Same as expts 13, 14, 15.

5

6

7

Mating mixtures: ♂ 4 ml + ♀ 12 ml.

8

9

0

Order of seeding flasks: Gal₅, Gal₂, Gal₁, Gal₃.

1

Times: 0', 18', 22', 25', 26', 28', 30', 32', 34', 40'.

22'

2

3

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

1

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4

5

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7

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0

1

2

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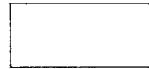
9

0

1408/16

19

REF:



19

June 22, 1958.

REF:

1408/17

1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	----

1 Hfr₂ timing of Gal_{1,2,8}
2
3
4

5 Same as exps 13, 14, 15, 16.
6

7 Mating mixtures: ♂ 4 ml + ♀ 12 ml.
8

9 Order of feeding flasks: Gal₈, Gal₁, Gal₂.
0

1 Timing 0', 18', 22' ^{20'} ✓, 24', 26', 28', 30', 32', 35'.
2

3 Note: Gal₁ at 22' is actually 23'.



19

June 23, 1958.

REF:

1408/18

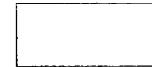
1 2 3 4 5 6 7 8 9 10
H₂O timing of Gal₂, Gal₃, Gal₆, Gal₉
same as expts 13, → 17.

Mating mixtures : 15 ml ♀ + 5 ml ♂,
sampling at times : 0', 20', 22', 24', 26', 28', 30',
for Gal₉ also 35', 40', 50'.

Order of reading : 2, 4, 6, 9

Note : Suspension of Gal₉ is granular, and slightly
less conc. than others in spite of using 4 tubes
resusp. to 24 ml. (4/3 conc.)

Time 22' of Gal₂ seems very thin, possibly
amount measured out of flask was spilled in
ice bath?



19

June 25, 1958

REF:

1408/19

1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	----

1

2 Hfr timing of Gal₂, Gal₅, Gal₇, Gal₈.

3

4 Same as exp 13-18. 16/10' rotation. Gal₅ slightly less turbid
5 than others.

6

Mating mixtures: 5 ml ♂ + 15 ml ♀.

7

8

9

0 Sampling at times: 0', 20', 22', 24', 26', 28', 30'.

1 Order of seeding 2, 5, 7, 8.

2

3

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

1

2

3

4

5

6

7

8

9

0

22/8 and 26/2 may have been exchanged
at mating?

19

June 27

REF: 1408/20

1

2

3

4

5

6

7

8

9

10

Hfr₂ timing of Gal, Try.

ORC cultures of W 3870, W 3908, W 4066/1 (Try-S^RGal-).

Refreshed, 1 ml + 7.5 ml for 1 hr growth at 37°C, resusp. in 2 ml (1:10 dilution). Crosses: 3870 x 3908; 3870 x 4066 - UV Gal- W 4076

Mating mixtures, in flasks: 2.0 ml ♂ + 6 ml ♀ = 1:3.

Samples: 0.2 ml + 1.8 chilled H₂O, ^{found standard} at every time: series A

if plated directly (.05 ml), if further diluted: series B

Schedule:

Time	D(SmB ₁) 4, S Gal SmB ₁	M Gal SmB ₁ , Try
------	--	------------------------------

♀ Control

A B

A B C

0

1/10

1/10

10'

1

20'

1

22

1

24

1

26

26'

1/20 = A 1 + 1

28

28'

1/40 1 + 3

30

30'

1/80 1 + 7

33

33'

1/200 .5 + 9.5

36

1/100 = A 1 + 1

39

1/400 1 + 3

42'

1/500 .2 + 9.8

42

1/1000 1 + 9

45

1/2000 .5 + 9.5

48

1/4000 25 + 9.75

51'

1/1000 .1 + 9.9

51

1/8000 125 + 9.9

54

1/16000 .1 + 9.9

57

1/32000 .1 + 9.9

60'

1/64000 .1 + 9.9

60'

1/1000 .1 + 9.9

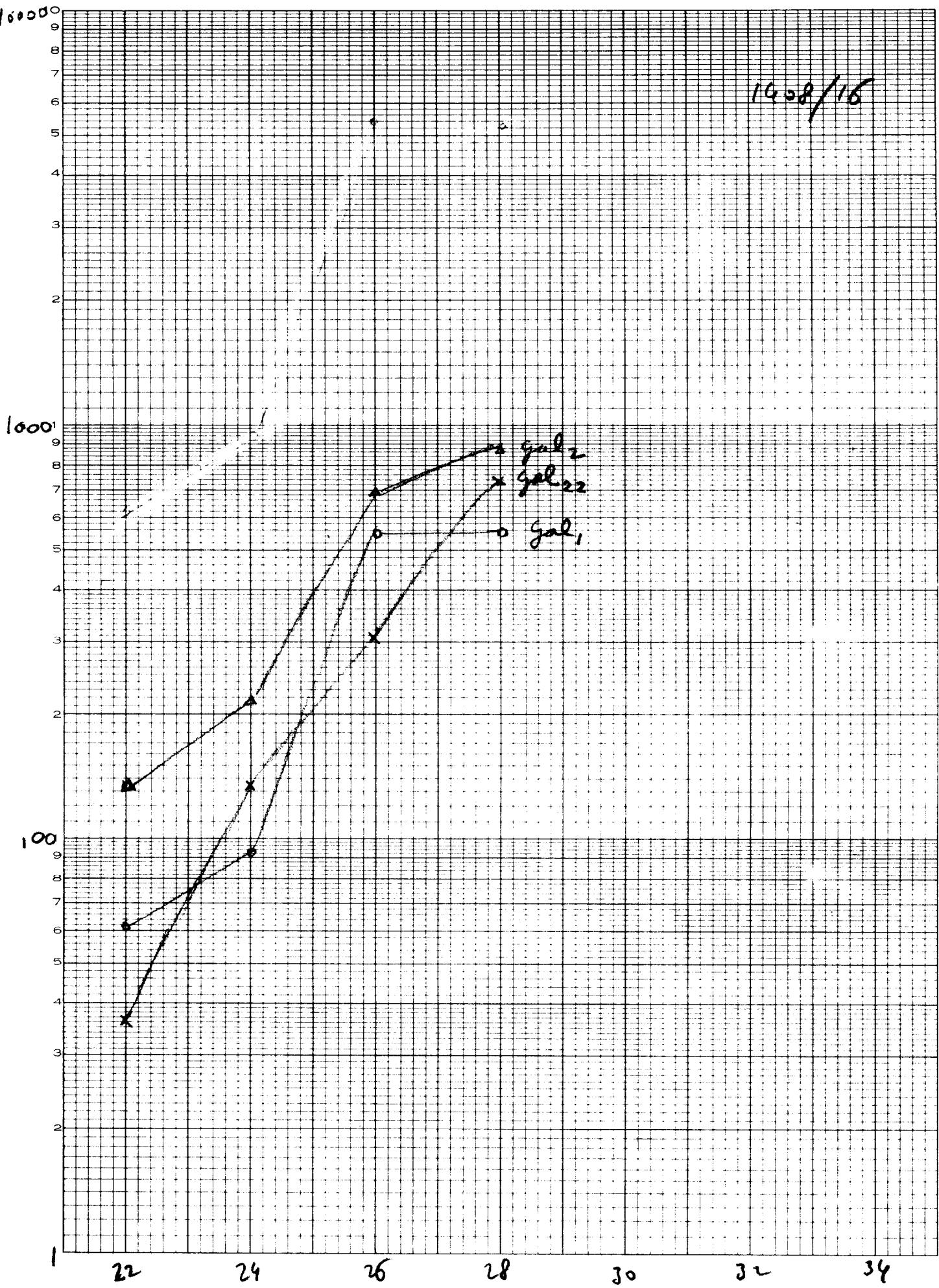
0/60 #

-

0/60

0/60 : control of plate recombination with parents kept in waterbath throughout the exp. (60').

Note: 51' missed. 33' was 40" late.



DATE: 4. 16. 54.

REF: 1409-5.

DATE:

4/18/58.

REF:

1409-2

1	2	3	4	5	6	7	8	9	10
ACTION OF ENZYMES ON MATING ♂♂ X ♀.									

3060 ♂ (2 ml + 10 ml L₂, 2'30' incub.), centrifuged, concentrated
10x in I₂ + 3064 ♀ conc. 36x in I₂.

0.3 + 0.3 ml in waterbath for 20', then 0.1 ml added to
1 ml of :

- | | |
|---|--|
| A | Chymotrypsin 1 mg/ml in I ₂ |
| B | Lysozyme, " |
| C | RN-ase " |
| D | DN-ase " |
| L | control, I ₂ medium. |

40' further incubation after addition to enzyme, then

~~0.02 ml~~ 0.1 + 10.0 DW → { 0.1 ml
on min st B,
0.01 ml

30 Protoplast suspensions: same as those used for exp. #405-5
before diluting 5x.

Plate counts: mm

	A	B	C	D	L
0.1	too many, ss	~	~	~	
0.01	113	43	62	74	93
Galt	0/48	2/43	0/47	0/42	1/50
Lact	7/48	5/43	5/47	1/42	6/50
T'	19/48	18/43	31/47	16/42	23/50

1410

DATE: 4/18/58 -

REF:

1410

1	2	3	4	5	6	7	8	9	10
					INTERRUPTION AND DIPLOIDS.				

2323, 2735, see overnight rotated cultures, mixed in equal amounts, pulse of 8', then diluted 1/200 in warm bath, further incubation: 20'; 40'; 60' in water bath

After such times:

0.1	\rightarrow	min B,	{	Hyphates 1: Stac NC!
$\frac{1}{10}$ 0.1	\rightarrow	"		$\frac{2}{2}: \overline{\text{Stac}}, \overline{\text{StacB}}, (\text{P}21)$
$\frac{1}{10}$ 0.01	\rightarrow	"		B Lac.

N.B. Numbers meaningless on account of smearing and poor scores on lac. (Liberates casein.)

D(B ₁) total	20' [A]	40' [B]	60' [C]
	288	412	181
Stac B ₁	9...29	—	52...80
Stac B ₁	34...42	—	38...42

$(\frac{1}{10} \cdot 1 \text{ ml})$.
Lac⁺

... due to mycelia

Lac + 15%
+ checked
4/22

216)

interrupted only by plating.

50

DATE:

REF: 1410

DATE:

22/4

REF:

1410/2

1	2	3	4	5	6	7	8	9	10
Experiment 1410/1 repeated.									

Platings of $\frac{1}{10}$ dil in DW of 0.1; 0.02 on min B,
0.1 on S' lac B, S' Gal B,

Note: Is plating interruption sufficient with 2323?

Frequency of Gal +青山 unaffected by plating time.
in exp 1410-1.

S' Gal willing for 40'.

Counts:

	20'	40'	60'
D(B ₁)	20'	40'	60'
$\frac{1}{10} 0.1$	26	111	35
0.02	2	4	6
S' lac B, 10 lacys			



19 30th April 1958.

REF:

1410/3

	1	2	3	4	5	6	7	8	9	10
--	---	---	---	---	---	---	---	---	---	----

1 1217 X 3064. DRC.
2
3
4
5
6
7
8
9
0

3 X conc. in saline, .05 on plate of min B.
3, 5 ml.
1217 Control: 0 colonies.

Colonies streaked on EMB lac.

DATE:

4/21/58

REF: 1411

1

2

3

4

5

6

7

8

9

10

COLCHICINE - Effect on mating

Solution of colchicine 1% in distilled water, non sterile, stored at -20°.

3060, 3064 overnight rotated cult:

0.1 ml. to 0.8 ml. Penafay + 0.1 H₂O (control)*
 " " + 0.1 colchicine, solution 1%.

1st rotation -

C₀: mixed equal amounts of colchicined broths, + 0.1% ml. colchicine / ml.

I: mixed equal amounts of (Control)* broth, 0.1 ml water added per ml.

C: mixed equal amounts of (Control)* broth, 0.1 ml water added per ml.

incubation in water bath, then dilution 1/100 in broth, incubation

20', then: $\frac{1}{10}$ DW → 0.05 min St B,

↓
 $\frac{1}{10}$ DW → same

↓
 $\frac{1}{100}$ DW → 0.05 Blac.

CROSSES

B Lac

Lac + Lac -

40

C₀
=

$\frac{1}{10}$ $\frac{1}{100}$

6 17

I
=

69

7

6 14

C
=

146

14

6 15

50

Conditions. there is perhaps a small decrease in

No. of matings adding colchicine to the mating mixture (but not adding it in advance to the cultures: adaptive enzyme destroying colchicine?) -

April 26 1958

REF: 1401-2.

1	2	3	4	5	6	7	8	9	10
ORE =	W 3060 (overnight)	3X	10 ml \rightarrow 3					.2	
overnight	W 3064S rotated	30X	10 ml \rightarrow 0.3 ml.	in fresh porc assay.				.3	mixed.
rotated									
cultures									
	3	prewarmed cultures mixed in a 125 ml flask. After 60 seconds add							
	4	19.6 ml prewarmed broth for 1:20 dilution. Mix gently in flask.							
	5								
	6	A7 Add 1 ml sample to prechilled tubes in CO ₂ -acetone bath.							
	7	A1 Dilute 1 + 3 ml 20% glycerol and freeze. = glycerol-freeze.							
	8	Dilute 1:250 in broth, incubate 15 minutes. Chill in ice bath.							
	9	B1 Sample 1+3 ml glycerol & freeze.							
	10	Blend 30 seconds.							
	11	C1 Glycerol-freeze							
	12	Dilute 1/9 in water and plate [D].							
	13	Incubate 45 minutes further.							
	14	E1 Glycerol-freeze	F1	dilute 1/9 in water and plate.					
	15	D and F plated .05 and .1 ml on DB, suc and B bac.							
	16	add 2 ml broth.							
	17	1.80	1.100	15 mins. chill.	Blend	1:9 H ₂ O	plate [D]		
	18	broth	broth	~2 ml	~2 ml	1.0			
	19	1 min pulse.		2 ml	0.2				
	20	A2	A1						
	21	freeze	glycerol						
	22	freeze	freeze						
	23	= 1/4	.1 / 9.9						
	24								
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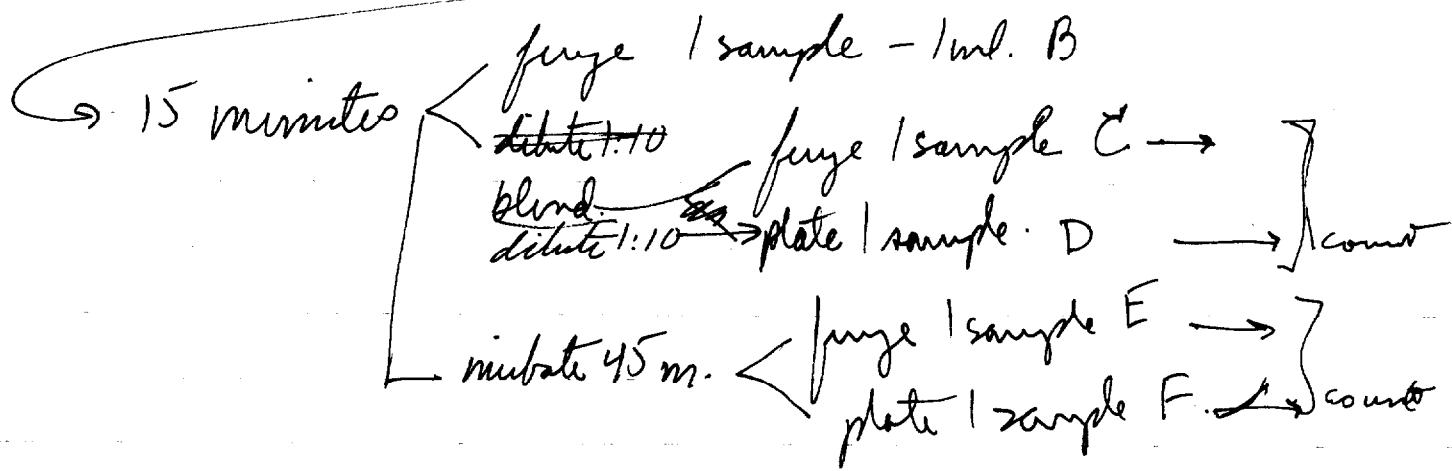
3060 x 3064.

(14/2)

5 1 ml samples:

1 ml.

Pulse 1 minute. 1:20 dilution: 1:200 dilution → 1 ml. sample.
= 20 ml. incubate



A - F. interruption by fusing as T_2^+ count.

B - F. interruption by bac ratio.

C - D | viability of zygotes.

C - E interruption by bac ratio

B, C, E vs D, F viability of zygotes as TL count.

C, E vs D, F viability of input.

storage; methods of thawing

FREEZE

DATE: 4/26/58,

REF: 1412

3060 & 3064 S.R.C. conc. 3 x batch 0.2 ml each in 50 ml flask volume

After 1' pulse add 1.9 ml batch from test tube. (1/50 dil)

Sample for further dilution (0.1 ml + 9.9 preserved body)

(1/5000)

(A₁) glycerol
freeze

tube: 0.2 + 0.6 glycerol 20%

15' incubation

chill
in icebath
2 ml

incubate
further 45'

(B)
glycerol
freeze
0.2 + 0.6

Blend

1+9 H₂O

(E)
glycerol
freeze
0.2 + 0.6

(F)
plate

(C)
glycerol
freeze
0.2 + 0.6

(D)
plate

40 Immediate platings: D, F on 1st B, 0.05 & 0.1

Counts:

0.1 0.05

D 12, 13 7, 9

F 45, 30 16, 25

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1	2	3	4	5	6	7	8	9	10
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C, E plated on 4/28/58 after thawing in water bath without further dilution (they are 2.5 x more concentrated than unwarmed sub, D, F). Plate on meatst B₁, 0.1 and 0.05 ml.

10

B thawed in water bath on 4/28/58, divided into B₁ & B₂.

B₁: incubated 45'; plate 0.1 & 0.05 on meatst B.

B₂: plated at once " "

20

Plate counts:

	B ₁	B ₂	C	E
0.1	19, 15	2, 11*	26, 27	30, 47
0.05	6, 8	4, 2	8, 9	31, 12
	-	-	-	-

30

Comparison between C & D: C, total 40 col. / 2.5 = 28

D, total 41

$$\text{survival } \frac{28}{41} = 67\%$$

E & F: E total 120 col. / 2.5 = 48

F 116 col

$$\text{survival } \frac{48}{116} = 40\%$$

B₁ & C B₁ 48 col.

C 70 col.

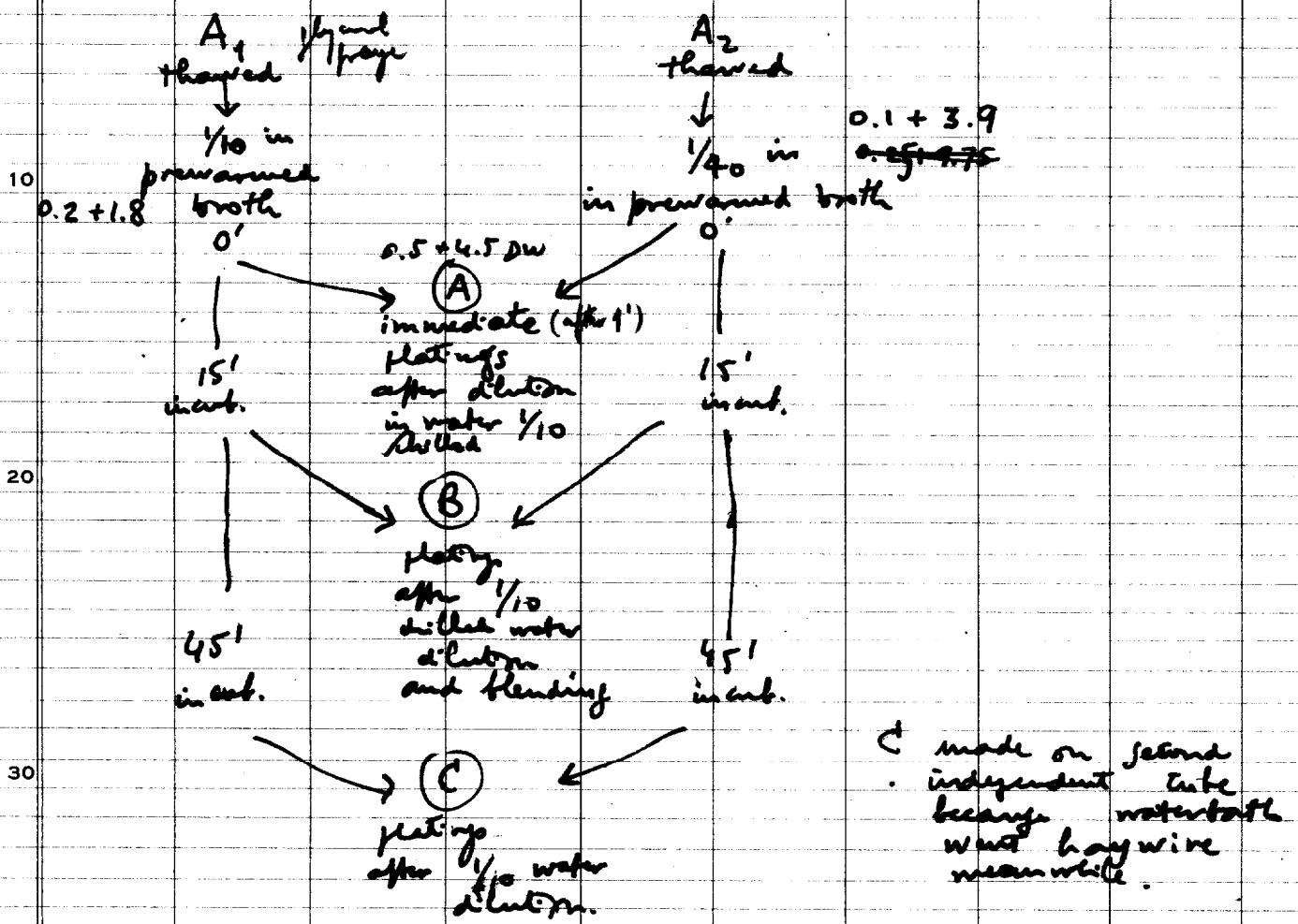
50

DATE:

4/29/58

REF: 1412

A₁ and A₂ tested for ability to resume mating.



Platings: .1 + .05 ml on min St Br.

Plate recombination controls-

Frozen parents (see exp 1412/2):

3060 [0.1 ml + 9.9 ml broth] \rightarrow [0.5 + 9.5 ml] \rightarrow 1/10 DW
30 x conc. from expon. culture, considered 20% of saturation.

3064 [0.1 ml + 9.9 ml milk] \rightarrow [5 + 95 ml] \rightarrow 1/10 DW
30 x conc. saturated culture.

From dilutions in water: platty with 0.05 + 0.05

0.01 + 0.01